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Original Research Article

Surface biofunctionalization of additively manufactured materials for implants with nano-thin polyelectrolyte multilayer coatings

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Abstract: Titanium-based additive manufacturing of medical implants has attracted considerable attention over the past decade due to numerous advantages over standard manufacturing. Regarding the surface modification and biofunctionalization of additive manufactured titanium materials carried out by the application of coatings, however, limited research has been reported so far. The interaction between the adherent tissues and the implant takes place at the interface between them, therefore the tissues response is strongly mediated and controlled by the surface properties of the implanted material. In this study we report on the surface modification of additively manufactured titanium materials with ultrathin polyelectrolyte multilayer coatings. The application of these coating with a thickness of only a few nanometers proved to be able to impart chemical homogeneity to the surface and allowed targeted modification of the hydrophilicity of the additively manufactured titanium materials without changing their macro-topography and bulk properties. An important and first-of-its-kind finding of the present study, which is being reported for the first time, is the adhesion strength of polyelectrolyte coatings to the surface of additively manufactured titanium materials that were found to meet the requirements of the ISO regulations for coatings, applied to metal implants. The non-cytotoxicity and high adhesion strength classify the polyelectrolyte multilayer coatings as very promising for application as coatings of additively manufactured medical devices.

I. Introduction

Additive manufacturing of materials, also called 3D printing, has attracted much attention and achieved significant progress in recent years, especially in the field of medical implant fabrication [1]. Among its advantages over machined implants is the potential to produce highly complex structured as well as customized devices that were not previously feasible and that can add significant functionality to already existing biomaterials [2].

Up to 80% of medical devices currently on the market have at least one type of coating material or surface treatment. Applying a coating to the surface of a medical device provides it with additional functionality and biocompatibility by mediating interactions between the implant surface and biological fluids and tissues. Surface properties such as free energy, roughness, morphology, stiffness, and wettability contribute to the biocompatibility of materials by influencing protein adsorption, which occurs immediately after implant placement in the body, and subsequent cell adhesion and tissue growth [3].

Due to the heterogeneity of the surface of additive manufactured (AM) materials, the need to apply a coating that ensures physical and chemical homogeneity of the surface is of utmost importance to improve bio-compatibility. Coatings reported so far for AM titanium materials are limited to hydroxyapatite, improving implant osseointegration [4,5], and given the greatly elevated surface area and susceptibility to bacterial infestation, a variety of silver nanoparticles-loaded [6] and antibiotics-loaded films imparting bactericidal activity [7].

The aim of this work was to add biomedical value to additively manufactured titanium (AMTi) materials

intended for application as dental implants through their biofunctionalization with ultra-thin polyelectrolyte multilayer (PEM) coatings. The main classes of medical coating materials offered on the world market are polymers (68%), ceramics (14%), and metals (12%). The majority of these coatings are single-component films whose properties are predetermined by their chemical nature and therefore with very limited potential for modification. PEMs are thin organic films obtained by alternating deposition of self-assembled monolayers of polyanions and polycations from polymer-salt aqueous solutions. We used PEMs because they provide the possibility of fine and controlled modification of all surface properties without affecting the surface micro-topology and bulk properties of the material to which they are applied [8]. Since a single polyelectrolyte layer is only 1-3 nm thin by depositing a certain number of polyelectrolyte layers, it is possible to adjust the coating thickness with nanometer precision. Termination of the coating with either a polycation or a polyanion layer allows switching of the surface charge, and post-treatment (e.g. thermal cross-linking) enables fine-tuning of the coating hardness and its propensity to degrade. In addition, PEMs provide very good adhesion to the material surface, which is of prime importance in the field of biomaterials. The selection of polyelectrolyte pairs used in PEM fabrication governs their physicochemical properties [8] and in consequence cellular adhesion to PEM-coated surfaces may vary from cytophilic to cytophobic [9]. So, inspired by these capabilities of PEM coatings, in this study, were applied one natural hyaluronic acid/chitosan (HA/Chi) and two synthetic polyacrylic acid/polyallylamine hydrochloride (PAA/PAH) and polystyrene sulfonate/polyallylamine hydrochloride (PSS/PAH) coatings on AMTi-surfaces and demonstrated how they impart the surfaces with their inherent individual physicochemical properties.

II. Material and methods

1 Manufacturing of AMTi-substrates

The additively manufactured titanium specimens (AMTi) were produced via powder bed fusion laser-based process (PBF-LB/M) using an industrial Lasertec 12 SLM machine from DMG MORI at the Laser Zentrum Hannover e.V. (Hanover, Germany). The machine was equipped with a 400 W fiber laser emitting at 1070 nm in continuous wave mode, with a minimum spot diameter of 35 µm. The powder used was Ti-6Al-4V grade 23 (ECKART TLS GmbH) with a predominantly spherical morphology and particle sizes ranging from 20.0 µm to 53.0 µm. The additively manufactured samples were subjected to ultrasonic cleaning and additional heat treatment to reduce manufacturing-related residual stresses and ensure a homogeneous and stable microstructure, in accordance with ISO20160 [10]. The heat-treatment was performed in a vacuum furnace at 1050°C for 4 h with conventional furnace cooling afterwards [11].

The fabricated samples were discs of 12 cm diameter and 2 mm thickness and cylinders of 26 mm diameter and 20 mm height. The average surface roughness was determined using optical profilometry (laser scanning confocal microscope VK-X1000 by Keyence).

2 Materials

Polyelectrolytes - poly(ethylene imine), PEI (750 kDa, 50%wt), polystyrene sulfonate, PSS (70 kDa), polyacrylic acid, PAA (100 kDa, 35%wt), and chitosan, Chi (50-190 kDa, 75%-85% deacetylated), all from Sigma Aldrich (Steinheim, Germany), poly(allylamine hydrochloride), PAH (120–200 kDa) from Alfa Aesar (Thermo Fisher (Kandel) GmbH), and hyaluronic acid, HA (360 kDa) from Lifecore Biomedical, LLC (Chaska), were all used as received. PSS, PAH, and PAA were dissolved in 0.5 M NaCl to a concentration of 2 mg/ml and adjusted to pH 7.0. HA and Chi were also dissolved in 0.5 M NaCl to a concentration of 1 mg/ml and adjusted to pH 5.5. PEI was dissolved in ultrapure water to a concentration of 2 mg/ml and adjusted to pH 7.0.

For the purpose of physicochemical characterization, the coatings were constructed on the AMTi-discs, on AMTi-cylinders, or on silicon (100) wafers (10 mm × 10 mm, CrysTec GmbH, Germany) pre-cleaned by successive ultrasonication in acetone and isopropanol (2 min each). Polished single crystalline silicon was used as a model substrate because it is almost perfectly smooth and has a well-defined homogeneous surface chemistry. For cell culture experiments, the same coatings were built inside sterile 24-well cell culture plates (Corning Inc., New York, USA).

3 Surface modification by PEMs

Three types of PEMs composed of five different polyelectrolytes were deposited on the surface of AMTi-substrates – PEI(HA/Chi)$_5$, PEI(PAA/PAH)$_5$ and PEI(PSS/PAH)$_5$. The coatings were assembled by applying the layer-by-layer (LbL) technology consisting of alternating deposition of polyanions and polycations adopting the protocol described in [12].

The thickness of the coatings was measured by ellipsometry (Sentech, Germany). One of the limitations of ellipsometry is that the surface of the sample under study has to be flat and smooth (ideally with roughness less than 50 nm) [13] therefore the thickness of PEMs was measured on model Si-wafers. The hydrophilicity was analyzed by static water contact angle measurements (DataPhysics, Germany) applying Young-Laplace fitting on model Si-wafers and on AMTi-discs. Scanning electron microscopy, SEM (Zeiss, Germany) was utilized for monitoring the surface topography.

The adhesion strength of PEM coatings to the surface of AMTi-materials was studied according to the ASTM F-1147-5 norm by an automatic pull-off adhesion tester

(PosiTest AT-A, DeFelsko). This test method measures the tensile force for adhesive or cohesive failure between two adhesive pull-off cylinders, one AMTi cylinder coated with PEM coating and one control smooth uncoated cylinder. Both cylinders were glued together with highly adhesive epoxide glue. Uncoated AMTi-cylinders were tested as a control.

All samples were prepared and tested in triplicate.

4 In-vitro cytotoxicity assays

The cytotoxicity of the PEM coatings was tested in triplicate in sterile conditions according to ISO 10993-5 by extraction of potentially cytotoxic substances. The extraction was carried out by incubating the PEM-coated substrates with cell culture medium (DMEM, Gibco, USA) with 10% FCS (Gibco, USA) with agitation at 37°C for 24 h. Cytotoxic latex and noncytotoxic polypropylene (PP) were also incubated and tested, as positive and negative controls. The extracts were used immediately for further biological tests.

The cell line used was the L929 fibroblast line and the cell culture medium was DMEM + 10% FCS + 1% penicillin/streptomycin (Gibco, USA). Cells were cultivated in 96-well plates for 24 h to form a sub-confluent cell layer. The old medium was discarded and replaced with the extraction medium. As suggested in ISO 10993-5, a series of dilutions of the extracted medium with fresh medium was prepared and tested. After an incubation time of 24 h, the vitality of the surviving cells was tested using the resazurin reduction assay.

III. Results and discussion

The three PEM coatings on the model Si-substrates were extremely thin, having a nanometric scale thickness of 15.6 ± 0.2 nm (for PSS/PAH), 5.8 ± 0.5 nm (for PAA/PAH), and 10.1 ± 0.1 nm (for HA/Chi). Consistent with the low thickness of the coatings, their average roughness was also minimal, in the range of 1.3 nm to 3.2 nm. Based on the findings in other studies, it can be assumed that the PEM thickness of AMTi-substrates is higher than that of Si-substrates. On the one hand, it has been shown that the thickness of the PEM increases with the roughness of the substrate [14], and on the other hand, that it depends on its chemical nature [15]. The thickness of $(PAH/PAA)_5$ multilayers on Ti-wafers was reported to be three times higher than that on Si-wafers, both substrates being single-sided polished and of similar roughness [15].

The first tool we used to demonstrate the successful construction of these just a few nanometer thin PEM coatings on AMTi-discs was through surface SEM imaging. Figure 1 shows representative SEM micrographs of uncoated and PAA/PAH-coated AMTi-discs. The surfaces coated with PSS/PAH and HA/Chi appeared analogous to that with PAA/PAH. The AMTi-discs had a complex microstructured surface with isotropically distributed irregularities resulting from the partially melted Ti-particles from the Ti-powder used in the 3D printing of the substrates (Figure 1A). The average roughness of AMTi-discs was estimated to be 14.64 ± 1.20 µm. Coating the AMTi-substrates with nano-thin and nano-smooth PEM coatings preserved their specific micromorphology, but smoothed the contours and removed loosely attached Ti-particles, imparting chemical homogeneity to the surface (Figure 1B). The PEM coatings adhered tightly and followed the surface of the AMTi-materials perfectly, with no defects such as scratches or delamination.

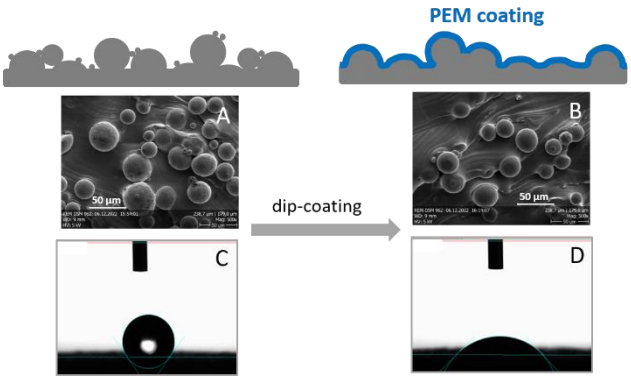

Figure 1: (A) SEM micrograph of a non-coated AMTi-disc, (B) SEM micrograph of a PAA/PAH-coated AMTi-disc, (C) water drop on an uncoated AMTi-disc, (D) water drop on a PAA/PAH-coated AMTi-disc.

The second tool used to demonstrate the successful construction of PEM coatings on AMTi-materials was by evaluating the change in hydrophilicity. For this aim, the static water contact angles of uncoated and PEM-coated AMTi-discs were measured and compared (Figure 1C,D). The data are summarized in Figure 2 and show that the uncoated AMTi-surfaces are moderately hydrophilic with a water contact angle of 67 ± 3°. Coating AMTi-substrates with HA/Chi coating preserves their hydrophilicity level, while PAA/PAH coating significantly increases their hydrophilicity (contact angle 23 ± 3°), and PSS/PAH coating makes them superhydrophilic with a contact angle close to zero. This is a proof that application of just a few nanometers thin PEM coating allows for purposeful modification of the hydrophilicity of AMTi-materials by choosing the suitable polyelectrolyte pair. Surface hydrophilicity, on the other hand, is one of the main surface characteristics moderating the adsorption and conformation of biomolecules (mainly proteins) on the surface, which in turn tunes the adhesion, proliferation, differentiation and migration of cells and microorganisms [16].

Surface roughness is well known to contribute to material wettability, so we also evaluated the contact angles of PEM coatings on smooth model Si-wafers (Figure 2). All PEM coatings on Si-wafers were hydrophilic with a water contact angle between 50° and 65°. Increasing the surface roughness had a contradictory PEM-dependent effect on the contact angle.

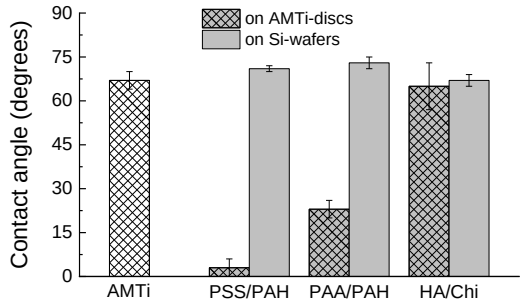

Figure 2: Average water contact angles of the noncoated AMTi-surface (white hatched column), PEM-coated AMTi surfaces (gray hatched columns), and PEM-coated Si-wafers (gray columns).

Wenzel's basic rule accounting for the effect of surface roughness on wettability states that the apparent contact angle of a smooth hydrophilic surface decreases with increasing surface roughness [17] and the PSS/PAH- and PAA/PAH-coated samples satisfy the rule of Wenzel, demonstrating a dramatic increase in hydrophilicity with surface roughness, reaching even superhydrophilicity (Figure 2). In the case of HA/Chi-coated samples, however, the same contact angle was found on the rough AMTi- and smooth Si-substrates. Other experimental exceptions to Wenzel's rule have already been published. As an example, the plasma-treated and roughened tissue culture plate (with an average surface roughness of 2.365 µm) showed a significantly higher water contact angle than the original untreated tissue culture plate (with an average surface roughness of 19 nm), regardless of whether the original smooth plate has a hydrophilic or hydrophobic character [18].

Adhesion of the coating to the implant body is the most important property among all mechanical properties that controls the performance and functionality of a coated implant. Delamination of the coating from the substrate leads to failed implantation and unstable long-term operation. We applied the pull-off (tensile) test, which measures the adhesion strength (defined as force per unit area) required to separate the coating from the substrate in accordance with the guidelines of the ASTM F-1147-5 standard, which evaluates the degree of adhesion of coatings to solid metal substrates or the internal cohesion of a coating in tension normal to the plane of the surface.

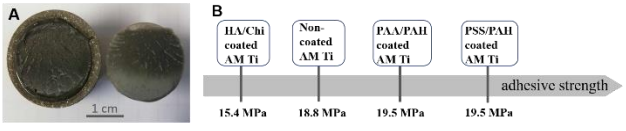

Figure 3: Results of the pull-off adhesion test for measuring the adhesion strength. (A) Adhesive pull-off cylinders after application of the pull-off test (left – the tested AMTi-cylinder coated with HA/Chi coating; right – the control smooth uncoated cylinder. (B) Overview of adhesion strength of various systems tested to ASTM F-1147-5, arranged in order of increasing adhesion strength.

The tensile load applied to detach the uncoated AMTi-cylinder from the control cylinder, also known as adhesive strength, was 18.8 ± 0.1 MPa (Figure 3B). The adhesive strength of the HA/Chi-coated AMTi-cylinder was lower and that of the uncoated, but the adhesive strength of the PAA/PAH- and PSS/PAH-coated AMTi-cylinders was higher.

In all cases, the fracture occurred entirely within the adhesive glue layer, as shown in Figure 3A. In such a case, it can be concluded that the adhesive strength between the surface of the material and the coating is greater than the adhesive strength of the glue itself. Such adhesive breakdown is usually seen in high quality coatings and it is accepted practice to qualify such coatings as very strongly adhering [19].

According to ISO regulations for hydroxyapatite coatings, coatings applied to solid metal substrates require a minimum adhesion strength of 15 MPa to be suitable for implant applications [20]. All this classifies the PEM coatings reported here as very promising for application as coatings of additively manufactured medical devices. It should be emphasized that such data on the adhesion strength of PEM coatings to metal substrates as well as adhesion of any coating to AM-material have not been reported elsewhere so far.

The safety of the coatings for application as medical device coatings has been proven by cytotoxicity tests in accordance with the requirements of the regulatory standard ISO 10993-5 used to certify the safety of medical devices for clinical use.

Figure 4 shows the growth inhibition of L929 cell line exposed to a dilution series of extracts from the three types of PEM coatings, as well as positive (cytotoxic latex) and negative (non-cytotoxic PP) controls. Controls show as expected: 100% growth suppression by the cytotoxic latex at high concentrations, decreasing upon the dilution and 0% by the non-cytotoxic PP. According to ISO 10993-5 if the highest concentration of the extract from the sample reduces the cell viability with 30% or less, then the material is considered non-cytotoxic.

The growth inhibition test revealed that the cell culture medium extracts from the PEM coatings exhibited no cytotoxic activity (Figure 4). No release of toxic substances inhibiting the cell growth was detected. The relative share of cell mortality was 12% at maximum (for PSS/PAH multilayer at 100% extraction medium) which is considered non-cytotoxic in accordance with the guidelines of ISO 10993-5.

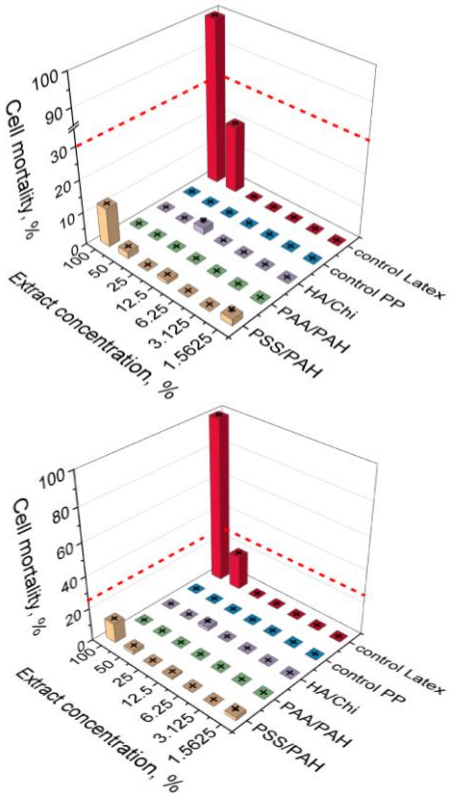

Figure 4: Cell mortality of L929 fibroblasts as a function of the concentration of the cell culture medium extracts from the three PEM coatings applied here – PSS/PAH (orange), PAA/PAH (green), HA/Chi (purple), and the controls - latex (red, positive control) and polypropylene (blue, negative control).

IV. Conclusions

We demonstrated that the application of PEM coatings on the surface of AMTi-materials had a strong effect on the surface hydrophilicity of the latter, which can be tuned in a wide range of values by using different natural or synthetic polyelectrolytes. The applied PEM coatings were only a few nanometers thin and with negligible roughness, but proved capable of providing chemical homogeneity on the surface of the AMTi-materials without affecting their specific macro-topography and bulk properties. An important and first-of-its-kind finding of the present study is the adhesion strength of PEM coatings to the surface of AMTi-materials, which is reported for the first time and found to meet the requirements of ISO regulations for coatings applied to metal implants.

We believe that this research will reveal a new broad way to modify the surface properties of AMTi-materials, thereby expanding their field of application not only in the field of medicine but also in other fields. Among the industries already benefiting from the amazing possibilities of additively manufactured materials are aerospace, automotive, energy, construction and medicine (especially implantology). But scientists and industry are so far only scratching the surface of what can be produced additively. The surface properties of any material play a crucial role as they determine not only the functionality but also the durability and safe use of the products, therefore most of the additive manufactured goods will need a surface coating and PEM coatings offer a wide range of options. Depending on the polyelectrolytes used in the production of PEM coatings, they can impart new properties to the materials such as well-controlled hydrophilicity or hydrophobicity, water and rust resistance, limited protein adsorption (dust resistance), hemocompatibility, cellular compatibility, antibacterial properties, etc. to the field of application. Our future plans include comparative studies of uncoated and PEM-coated AM-materials in terms of blood proteins adsorption as well as adhesion and proliferation of different cell types (fibroblasts, endothelial cells and osteoblasts). The effect of the level of surface microroughness of AM-materials with and without PEM coatings on the biological response is currently being investigated.

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Author's Statement

Authors state no conflict of interest.

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